FORTIFICATION OF SOFT DRINKS WITH PROTEIN FROM COTTAGE CHEESE WHEY

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ABSTRACT

Cottage cheese whey protein concentrates, prepared by preconcentration by ultrafiltration followed by gel permeation to remove low molecular weight materials, have the solubility, stability and flavor that make them suitable for fortification of soft drinks and related products. These concentrates are characterized by high levels of "available" lysine and by amino acid compositions indicating good nutritional value. Carbonated beverages prepared with conventional beverage ingredients and containing up to 1% by weight of the total beverage of added whey protein maintained clarity, color, and flavor during 203 days storage at room temperature. Spray dried whey protein concentrates were incorporated without adverse effects into commercial "ade" type powders. Clarity of 1% protein solutions at pH 2-3.5 was not impaired by heating for $6h\ \text{at}\ 80\,^{\circ}\text{,}$ but some structural change occurred since an average of 37% of the protein present precipitated on shifting pH to 4.7. Increased stability against heat denaturation under acidic conditions was conferred by some soft drink ingredients. Added sucrose reduced protein denaturation by 1/2 but sodium saccharin had no effect. The type of acid used also altered protein denaturation rate. While properly isolated whey protein concentrates have functional properties necessary for soft drink fortification, feasibility of use will depend upon cost.

INTRODUCTION

The soft drink industry in the United States can serve as a model of successful merchandizing as Americans of all ages and

walks of life consume its products. Most of these are purchased along with the family's weekly food requirements; in 1975, 2.45% of the average food dollar spent at the supermarket went for soft drinks (Anon., 1976). Although soft drinks contribute only about 4.3% of the caloric requirement of the population and therefore should be of little national concern, many nutritionists consider them to be a type of dietary pollutant because of their strong appeal to the young. As more nutritious beverages such as milk and fruit juice are replaced in the diets of children and teenagers, nourishing materials such as calcium and protein are replaced by carbohydrate calories.

Consequently, the soft drink companies have been under great pressure from consumer advocates and nutritionists to improve not only the nutritional quality of their product (Nader, 1972) but also the quality of their advertising (Morris, 1973). Steps which can be taken to alleviate these criticisms would be either to fortify the familiar beverages with valuable nutrients without detectable change in flavor or appearance or to develop new types of beverages for the snack trade.

The U.S. Department of Agriculture has concerned itself for many years with the development of foods high in protein. The unique functional properties of proteins isolated from cheese whey make them suitable for the fortification of carbonated beverages.

Traditionally, whey, the byproduct of cheese manufacture, has been fed to pigs or dumped into streams and municipal sewage systems. Because the disposal of whey is a growing problem in the United States due to the large increase in cheese production in recent years (USDA, 1977), increasing concern over pollution has led to stricter environmental controls. Consequently, research efforts have been expanded toward developing new uses for whey, particularly acid whey, which is more difficult to process because of its high lactic acid content ($\underline{\text{Table 1}}$). Since acid whey is now being wasted and is a serious pollutant in some areas, a soft drink fortification program could yield benefits to both snack drink consumers and cheese manufacturers.

USE OF WHEY IN BEVERAGES

The manufacture of both alcoholic and nonalcoholic beverages from whey has been attempted for many years, particularly in Europe (Holsinger et al., 1974). As early as the seventeenth century, medicinal properties were ascribed to whey; this led to the growth of "whey houses" designed for the treatment of a variety of human ailments, especially those related to digestion.

Some commercial success has been achieved with the development of drinks containing whey components. Perhaps the most successful

TABLE 1
Composition of whey solids^a

	Cheddar whey (sweet)	Cottage cheese whey (acid)
% Total protein	11.5	11.4
% Lactose	74.4	66.8
% Ash	7.4	10.2
% Lactic Acid	< 1.0	9.6
% Fat	2.7	< 1.0
pH	6.5	4.7

^aHolsinger (1976).

is Rivella, a sparkling, crystal clear herbal infusion in deproteinized whey that appeared on the market in Switzerland in 1952 (Susli, 1956). This product is currently sold in most of Western Europe, being promoted as a therapeutic tonic. Rivella resembles ginger ale in flavor and appearance; it has been pasteurized and must be refrigerated after opening.

Alcoholic beverages produced from whey include beer-like drinks that have been marketed with some success in Russia and a champagne-like product in Poland (Holsinger et al., 1974). In the United States, Yang et al. (1975) developed a process for making wine from whey which is undergoing commercialization trials in California. By adding dextrose to the whey, they use a non-lactose fermenting organism to produce the alcohol.

A conventional type nonalcoholic orange flavored carbonated beverage fortified with 1.5% whey protein was test marketed in Brazil in 1971 (Anon., 1973). Since the whey protein concentrate used contained milkfat, lactose, and whey salts, these were also present in the drink. This product, although resembling the carbonated soft drinks that dominate the American beverage market, did not advance beyond the test market stage.

RESEARCH TOWARD SOFT DRINK FORTIFICATION

Protein fortification offers a means of increasing the nutritive value of soft drinks. The possible magnitude of a fortification program using protein isolated from cheese whey may be estimated from a consideration of the volume of soft drinks consumed in the United States. In 1976, 459 8-oz. bottles of soft drinks were consumed per capita, representing an estimated nine billion

dollars in sales (Anon., 1977). In addition, beverage powders were consumed after reconstitution at a rate of 7.7 gallons/capita, representing an estimated one billion dollars in sales (Anon., 1977).

Acid cheese whey represents about 18% of the total whey production (USDA, 1977). The estimated amount of recoverable protein in this wasted byproduct is 64 million pounds. A price quotation of \$1.50 per pound has been made for a whey protein isolate containing 75% protein (Stauffer Chemical Co., 1977). If a market existed for protein recovered from all the whey produced annually, this could represent a sizeable potential income for protein processors.

Considering the volume of soft drinks manufactured in the United States, the potential amount of acid whey protein available and its projected price, one can calculate that 13% of the total soft drink production in the United States could be fortified with 1% cheese whey protein at an added materials cost of about one cent per 8-oz. bottle. Fortification at this level would increase the nutritional value of soft drinks. Therefore, research was carried out to determine if proteins isolated from cottage cheese whey, regardless of cost, had functional characteristics that made them suitable for soft drink manufacture (Holsinger et al., 1973a). Fortification with only whey proteins represents a different approach than that taken by previous researchers. The development of new methods to isolate the whey proteins from the lactose and salts made this approach possible.

A large-scale process was developed to permit the isolation of undenatured proteins from cottage cheese whey (Holsinger et al., 1975). The whey proteins were preconcentrated by ultrafiltration at pH 4.7; low molecular weight materials were then removed from the preconcentrate by gel permeation on Sephadex G-25. The high protein eluate obtained was condensed under vacuum and spray dried. In order to produce a highly soluble protein, centrifugal clarifiers were used to remove residual milkfat and insoluble material formed during the purification steps. The isolates produced contained 75-90% protein after dehydration. Whey proteins are exceptionally rich in the essential amino acid lysine; isolates prepared by this procedure had over 90% of the total lysine in a nutritionally available form as measured by chemical means (Holsinger et al., 1973b). This was one indication that the quality of the dried products was excellent.

Carbonated soft drinks were formulated and fortified with a whey protein concentrate containing 81.4% protein, 10.2% lactose, and 1.5% ash (Table 2). Solid carbon dioxide was used to achieve carbonation.

TABLE 2

Composition of protein-fortified carbonated soft drinks,

percent by weight^a

	Flavor			
Ingredient	Strawberry	Orange	Lemon	Lime
Sucrose	12.0	14.0	13.0	13.0
Flavoring	0.37	0.37	0.37	0.37
Citric acid	0.37	0.185	0.74	0.74
Protein	1.0	1.0	1.0	1.0
Water	86.26	84.44	84.89	84.89
Carbon Dioxide Volumes pH before carbo- nation	2	1	1	1
	2.50	2.66	2.35	2.46

^aHolsinger et al. (1973a).

Beverages fortified with 1% whey protein maintained clarity and color over one year of storage in colorless glass bottles on shelves and in glass-fronted cabinets in the laboratory. However, compared with a freshly made control, the lime-flavored drink had faded slightly in color after 203 days of storage, but the flavor remained unchanged; after one year, a slight stale whey flavor was tasted in the fortified product.

Carefully prepared spray dried whey protein concentrates are also well suited for the fortification of the sweetened powders that are dissolved in water to form the popular "ade" type drinks. Seven flavors of these products were fortified with 0.5% and 1.0% whey protein, and the reconstituted beverages along with unfortified controls were then submitted to trained dairy products judges. One flavor was tested per panel. Using the 9-point hedonic scale of Peryam and Pilgrim (1957), the judges were asked to rate acceptance; on this scale, the higher the number, the more acceptable the flavor (Table 3).

Analysis of variance (Larmond, 1970) showed that at the 5% confidence level, only the scores of the cherry and lemon-lime flavored drinks fortified with 1% whey protein deviated significantly from their controls. However, no dislikes were registered, even though some products neared the indifferent response, a score of 5.0, at the highest protein concentration. Even though fortification with 1% whey protein was not readily detectable in some

 $\begin{array}{c} \text{TABLE 3} \\ \text{Organoleptic evaluation of protein fortified noncarbonated} \\ \text{soft drinks}^{a} \end{array}$

	Average hedonic ranking				
Flavor	Control	0.5% Protein	1.0% Protein		
Cherry Grape Tart Lemon Lemon-Lime Orange Raspberry Strawberry	7.0 7.2 5.7 7.2 6.2 6.3 6.5	6.5 6.8 5.2 6.5 5.9 6.7 5.9	5.5 ^b 6.3 5.5 _b 6.2 ^b 6.0 6.4 6.0		

^aHolsinger et al. (1973a).

instances even by experienced judges, it might be difficult to produce soft drinks with protein levels approaching the 3.3-3.9% protein found in fluid milk (Posati and Orr, 1976).

An unpublished report suggests that the type of acid used in the soft drink formulation could have an effect on the detection of off-flavors brought about by fortification with whey protein (Berger, 1977). Citric acid was used in the formulation of the beverages studied by Holsinger et al. (1973a). Malic acid, which is known in the food trade for its ability to mask and smooth out off-flavors (Gardner, 1966), has reportedly effected considerable flavor improvement when used in whey drink formulations. As malic acid is an ingredient in many present-day "ade" powders, fortification with whey proteins above the 1% level may be possible.

WHEY PROTEIN BEHAVIOR IN THE SOFT DRINK SYSTEM

Little information is available about the solubility and stability of the mixed whey proteins at the low pH values characterizing most carbonated beverages. Guy et al. (1967) examined the denaturation of cottage cheese whey proteins by heat but did not investigate the behavior of protein solutions of pH below 3.4.

While empirical studies showed that soft drink fortification with whey protein isolates was possible, more fundamental data were needed to make commercialization feasible. Holsinger et al.

^bSignificantly different, P < .05.

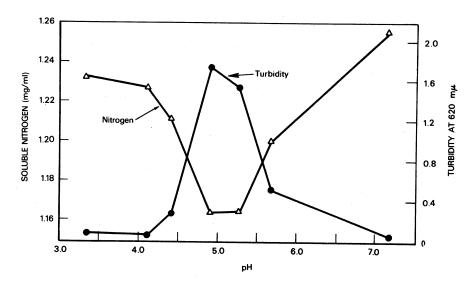


Figure 1. Change in solubility and turbidity of a high protein isolate from acid cheese whey with pH (Holsinger et al., 1973a).

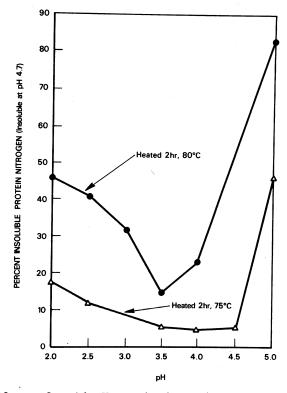


Figure 2. Effect of acid pH on the heat denaturation of a 1% whey protein solution (Holsinger et al., 1973a).

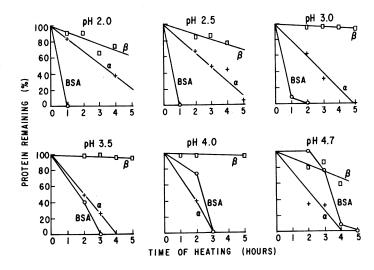


Figure 3. Effect of pH on concentration of whey proteins remaining after heating at 80°. BSA = bovine serum albumin, β = β -lactoglobulin, α = α -lactalbumin.

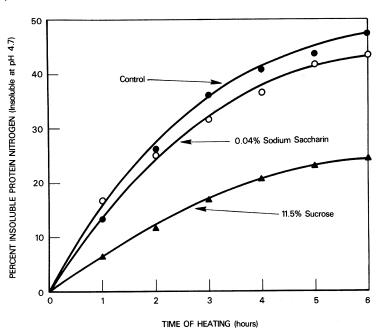


Figure 4. Effect of added sucrose and sodium saccharin on protein stability at 80° and pH 3.3 when acidified with citric acid (Holsinger et al., 1973a).

(1973a) studied the solubility and stability of whey proteins under acid conditions to provide some of the additional information required.

The solubility and turbidity of a whey protein concentrate dissolved in water varied over the pH range from 3.5 to 7.5 (Figure 1). Decrease in solubility was accompanied by a sharp increase in turbidity around pH 4.7, the region of the proteins' isoelectric points. Quite unexpected was the clearing of the protein solution in the more acid pH ranges typical of carbonated soft drinks. These results explain beverage clarity when whey proteins were added to the soft drink formulations.

Heating at elevated temperatures indicated that beverage clarity could be maintained over long periods of time (Figure 2). The whey proteins were most resistant to thermal denaturation at pH 3.5. As the pH of most commercial carbonated drinks is near this value, solubility changes on storage should be of little concern.

Prolonged heating at different pH's produced changes in the relative concentrations of the individual whey proteins in the mixed system (Figure 3). Heated samples containing equivalent amounts of protein were examined electrophoretically and quantified by densitometry. An unheated sample served as the control. The curves show that, as the pH became more acid, bovine serum albumin (BSA) became increasingly less heat stable, while the heat stability of α -lactalbumin increased. At pH 3.5, BSA and α -lactalbumin showed approximately equivalent heat stabilities. β -lactoglobulin, comprising about 70% of the whey proteins, seemed little affected by heat in the pH range 3-4.

After one year of storage at room temperature, chemical changes had occurred in the fortified carbonated drinks. When bottles of a lime-flavored carbonated drink were opened for tasting, no sediment was visible. However, 9% of the protein precipitated when the pH of the drink was raised from 2.5 to 4.7. During the storage period 84% of the sucrose present in the beverage had inverted. In spite of the presence of the reducing sugar formed, only 3% of the total lysine in the protein had been destroyed and 95% of the lysine remaining was nutritionally available as measured by chemical means.

Many of the soft drink ingredients themselves stabilize the added protein against heat damage. Sucrose, a popular sweetener in soft drinks, retarded the heat denaturation of whey proteins (Figure 4). After heating for 6h at 80°, the sucrose containing solution had only half the amount of denatured protein found in the unsweetened control. Sodium saccharin, however, conferred

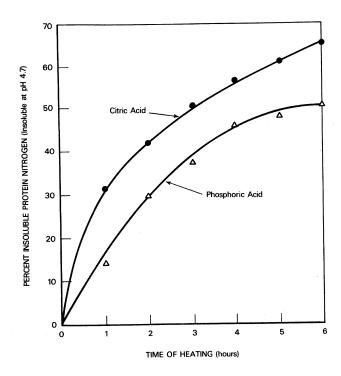


Figure 5. Effect of type of acid on protein stability at 80° and pH 2.68 (Holsinger et al., 1973a).

little protection against heat denaturation. At the present time, there is no information available about the behavior of whey protein concentrates in the presence of the high fructose corn sweeteners that are finding increased use in many soft drink formulations.

The type of acid used in the soft drinks also influences protein stability. The rate of denaturation of the whey proteins in phosphoric acid solutions of pH 2.68 was less than that noted in solutions brought to the same pH by addition of citric acid (Figure 5).

The whey proteins showed good heat stability when heated at 80° in two popular commercial soft drinks (Figure 6). The proteins were less stable in the cola beverage, which had a pH lower than the point of greatest heat stability. The pH of the citrus beverage was very close to the point where the proteins are most stable to heat denaturation. The electrophoretic behavior of the individual whey proteins in the two beverages (Figure 7) was similar to that observed in the samples previously described (Figure 3). This suggests that the presence of flavorings and colorings

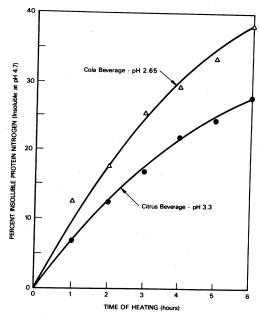


Figure 6. Protein stability in commercial soft drinks heated at 80° (Holsinger et al., 1973a).

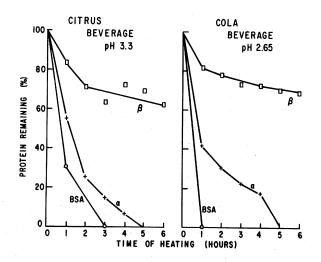


Figure 7. Concentration of whey proteins remaining in two commercial soft drinks after heating at 80°. BSA = bovine serum albumin, β = β -lactoglobulin, α = α -lactalbumin.

affected the protein stability only slightly, if at all, in these particular products.

From these results, Holsinger et al. (1973a) concluded that most soft drinks of pH below 4 could be successfully fortified with undenatured cheese whey proteins. Precipitation problems might be encountered with the protein fortification of those drinks containing natural fruit juices high in tannins; some caramel colorings also act as protein precipitants. However, with only slight formula modifications, a stable system should be attainable.

The success of a fortification program of this magnitude is strongly dependent upon cost. Several commercial concerns are currently producing high quality whey protein isolates of varying protein content. Consumer interest in improved nutrition could provide the necessary impetus leading to the commercial production of protein fortified soft drinks.

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